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GENETICAL STUDIES ON ÆNOTHERA. IV

II

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3. HYBRIDS OF *grandiflora* $B \times biennis$ D IN THE F_2 GENERATION

In my last paper (Davis, '12a, pp. 392-406) there was described an especially interesting F_1 generation, culture 11.35, of the cross *grandiflora* $B \times biennis$ D . The *biennis* male parent of this cross (Davis, '12a, pp. 385-389, Figs. 1-3) was of a race with the stem coloration characteristic of *Lamarckiana*, i. e., the papillæ or glands at the base of long hairs were colored red on green portions of the stem. The *grandiflora* female parent bears the same type of papillæ, but they follow the color of the stem and therefore lack the red over green portions of the stem. The *biennis* parent then presented a character, the red coloration of the papillæ, that might be expected to be present or absent in the F_1 , and to present an alternative inheritance in the F_2 generation.

It will be remembered that in the F_1 generation of this cross, *grandiflora* $B \times biennis$ D , consisting of 180 plants, two sharply contrasted classes appeared (Davis, '12a, p. 395). Class I was represented by 12 plants which had the stem coloration of the *biennis* parent (red papillæ on green portions of the stem). Class II was represented by 168 plants with the stem coloration of the *grandiflora* parent (stems above clear green). Other peculiarities of these classes are described in the paper cited above, but we are concerned at this time chiefly with the behavior of this color character. The mixed conditions in this F_1 generation naturally suggested the probability that the male *biennis* parent was heterozygous with respect to the red coloration of the papillate glands and that it formed

two classes of gametes with and without the factor responsible for this character.

Although it is true that the form *biennis* *D* in later generations has been uniform as to the stem coloration described above it by no means follows that the original plant of 1910, which furnished the gametes of the cross, was homozygous for this character. I have already noted the fact (Davis, '12a, p. 386) that types occur wild similar to *biennis* *D* except for their clear green stems. Consequently the original plant may have been heterozygous with respect to factors for red papillæ and in my later generations I may have isolated a homozygous line.

Last summer I grew the reciprocal of the cross described above, *i. e.*, a cross *biennis* *D* \times *grandiflora* *B* which involved the same parent plants as in the first. The F_1 generation of 103 plants, culture 12.11, was brought to maturity and consisted of the same two clearly defined classes. Class I, consisting of 87 plants, presented the stem coloration of the *biennis* parent (red papillæ on green portions of the stem). Class II, consisting of 16 plants, presented the stem coloration of the *grandiflora* parent (stem above clear green). There was a disproportion of the numbers as in the previous case, but in the reciprocal cross the plants with red papillæ were in a large majority, 87:16, instead of being in a small minority, 12:168, present in the first cross. Other peculiarities of these classes were the same as in the first cross. Again the mixed conditions in the F_1 reciprocal cross suggested the probability that the *biennis* parent, in this case female, was heterozygous with respect to the red coloration of the papillate glands and that it also formed two classes of gametes with and without the factor responsible for this character.

The two classes of hybrids in the F_1 generation described above appear to present a phenomenon similar to the "twin hybrids" of De Vries ('07) which result when *Æ. biennis* or *Æ. muricata* are pollinated by *Lamarckiana* or by one of its derivatives (*e. g.*, *rubrinervis*, *brevistylis*

or *nanella*). Critics have pointed out that this behavior indicates that *Lamarckiana* is heterozygous or hybrid in character since it must form at least two different types of male gametes. De Vries apparently believes that the "twin hybrids" in my crosses show that the *grandiflora* parent is in a condition similar to that of *Lamarckiana* and that the "twin hybrids" are due to the mutations of *grandiflora*. My interpretation of the behavior is quite the opposite, for, as will be shown, the evidence indicates that the *biennis* parent, with respect to the characters concerned, is heterozygous and that the race of *grandiflora* is stable. If this is true the evidence does not indicate that the race *grandiflora B* exhibits with respect to these characters the habit of mutation as claimed by De Vries ('12, p. 30).

Among some 300 plants of *grandiflora* grown from wild seed and 200 more grown in isolated lines none have presented red-colored papillæ over green portions of the stem. All green-stemmed forms of *biennis* have proved perfectly true to this character. One of the best known types of green-stemmed *biennis* is the Dutch plant extensively grown by De Vries and Stomps, and this, as far as I know, is constant. Furthermore, all green-stemmed F_1 hybrids have in later generations proved constant to this form of coloration. There is thus much evidence that the absence of red in papillæ over green portions of the stem constitutes a homozygous condition. The type *biennis D*, as stated before, can not be distinguished in other respects from wild plants which lack the red coloration in their papillæ, and it seems probable that this assemblage is a mixed population in which some plants are heterozygous with respect to the character of their stem coloration.

Although I can not as yet present experimental proof that the red coloration of papillæ is a character dominant to its absence, we should expect this to be the case because anthocyan coloration is obviously a character in addition to that of the green and because its inheritance appears

to be alternative. It is on this hypothesis that I shall treat the red coloration of papillæ as a dominant character when for convenience employing a Mendelian notation in the accounts that follow.

It became a matter of interest to determine how representatives of Class I and Class II would behave in the F_2 when selfed and how they would behave when crossed reciprocally. Therefore I selected a plant, 11.35*m* (Davis, '12*a*, Figs. 6 and 7), as representative of Class I, and a plant, 11.35*a* (Davis, '12*a*, Figs. 5, 8 and 9), as representative of Class II, and according to my plan (Davis, '12*a*, p. 399) carried these in pure lines into an F_2 and also grew the crosses 11.35 *m* \times *a* and 11.35 *a* \times *m*. Furthermore, a large F_2 generation was grown from an especially interesting plant 11.35*La* (Davis, '12*a*, Figs. 10, 11, 12 and 13), also representative of Class II, which resembled *Oenothera Lamarckiana* closely in certain particulars. These cultures will now be briefly described.

1. *The F_2 Generation from 11.35*m*.*—From this plant, with red papillæ on green portions of the stem as in the parent *biennis*, the contents of one capsule, 413 seeds, were sown. The culture, 12.43, produced 180 seedlings, of which 166 plants were brought to maturity. Among these, 86 plants presented the stem coloration of 11.35*m* and the *biennis* parent of the cross, and 80 plants presented the stem coloration of the *grandiflora* parent.

Let us assume the formula for the *biennis* parent to be *Rr* (*R* standing for the presence of the factor responsible for the red color of the glands and *r* for its absence); *i. e.*, the *biennis* parent is held to be heterozygous for this character and to form two classes of gametes, *viz.*, *R* and *r*. Let us assume that the formula for the *grandiflora* parent with respect to this character is *rr*. The F_1 hybrid plant 11.35*m* would then be expected to have the formula *Rr* and to produce gametes *R* and *r*. These gametes in chance combinations should give F_2 hybrids in the proportions 1*RR*:2*Rr*:1*rr*, which would be a 3:1 ratio with respect to the appearance of the character *R* (red

papillæ). I lay no stress on the fact that in my small cultures the numbers were 86*R*:80*r*, but merely wish to note the point that in this F_2 generation two classes appeared sharply distinguished by the presence or absence of the character under discussion.

I was unable to differentiate in this F_2 other characters on the plants 11.35*m* and 11.35*a* associated with the presence or absence of the red papillæ (see Davis, '12*a*, p. 395). There was a wide variation in habit, leaf, form, inflorescence, flower proportions and flower size (petals 2.2–3.9 cm. long), a variation that seemed unrelated to the presence or absence of red papillæ. In this culture also appeared a group of 15 dwarfs, recognizable when young rosettes, which at maturity were from 5–6 dm. high, sparsely branched, and with a foliage of narrow leaves; 6 of these dwarfs had the stem coloration of the *biennis* parent (red papillæ) and 9 that of *grandiflora*.

Among the plants with red papillæ on the stems I selected an individual, 12.43*g*, which among my hybrids with the stem coloration of *Lamarckiana* most resembled that form. I shall make this plant the starting point of a pure line with the hope that in later generations I may find variants still closer to the *Lamarckiana* type which may be isolated by selection. Whether the plant is homozygous with respect to the red coloration of the papillate glands is a point to be determined by the next generation.

2. *The F_2 Generation from 11.35*a*.*—From this plant with the stem coloration of the *grandiflora* parent (papillæ green over green portions of the stem), the contents of one capsule, 432 seeds, were sown. The culture, 12.42, produced 165 seedlings of which 147 plants were brought to maturity. These presented uniformly the stem coloration of the F_1 hybrid plant 11.35*a* and of the *grandiflora* parent. It seems then safe to conclude that such a plant as 11.35*a* is homozygous as to its stem coloration with possibly the formula of a recessive (*rr*) lacking the factor that produces the red color in the papillate glands. This position is supported by the evidence from the much

larger F_2 generation grown from the sister plant of the same Class II, 11.35*La*, where 532 plants agreed in having this same type of stem coloration characteristic of *grandiflora*.

The culture was remarkable for the length and breadth of its leaves, which far surpassed that of the parents of the cross and for its general vigor. In these respects there was marked progressive evolution. The flower size, however, was below the *grandiflora* type, the petals ranging from 1.5 to 2.8 cm. long (those of *grandiflora* being about 3.3 cm. long). Since none of these plants appeared to present the possibility of developing the stem coloration of *Lamarckiana*, I have not considered it worth while to follow the family further.

3. *The Cross 11.35 m × a and its Reciprocal 11.35 a × m.*—These crosses were made to determine whether or not the peculiarity of the red glands with the other correlated characters was in any sense or degree sex-limited. Thus if these characters were carried by the male gametes from the plant 11.35*m*, the progeny of the cross 11.35 *a × m* should have the peculiarities of Class I, while the progeny of the cross 11.35 *m × a* should have the peculiarities of Class II. A behavior of this general nature has been described by De Vries ('11) in his paper on double reciprocal crosses.

From the cross 11.35 *m × a* the contents of one capsule, 276 seeds, were sown. The culture, 12.45, gave 143 plants which were brought to maturity. Of these, 50 plants presented the red-colored papillæ characteristic of 11.35*m* and of the *biennis* parent, and 83 had the coloration of 11.35*a* and of the *grandiflora* parent. On the hypothesis developed through the cultures previously described the plant 11.35*m* should have the constitution *Rr* and the plant 11.35*a* should have the constitution *rr*. The female gametes of 11.35*m* should then have been of two sorts (*R* and *r*), the male gametes from 11.35*a* should have been all similar (*r*), and the plants of the culture distinguished as 50*Rr* and 83*rr*. The expected ratio of the two classes

would be 1:1, provided that the female gametes R and r were formed in equal numbers and mated in equal proportions with the male gametes (r). It is at least clear from this culture that the factor for red glands (R) is in this case carried by a certain proportion of the female gametes and that the female gametophytes for the plant 11.35*m* must be of two sorts (R and r).

The plants of this culture, 12.45 (11.35 $m \times a$), failed to exhibit consistently the other differences associated with the presence or absence of red glands as illustrated by the two F_1 types 11.35*m* and 11.35*a*. There was a marked progressive advance over the parent species, *biennis* and *grandiflora*, in leaf size and general vigor, but not in flower size, the petals ranging from 1.5 to 3.2 cm. in length.

From the cross 11.35 $a \times m$ the contents of one capsule, 223 seeds, were sown. The culture, 12.44, gave 142 plants which were brought to maturity. Of these 23 plants presented the red-colored papillæ characteristic of 11.35*m* and of the *biennis* parent, and 119 had the coloration of 11.35*a* and the *grandiflora* parent. The proportions of these two types (23:119) is far from the expected ratio 1:1 on the hypothesis considered above, but it should be noted that the total number of plants in the culture (142) is small. The main consideration is, however, clear, viz., that the factor for the red papillæ is in this case carried by a certain proportion of the male gametes and that the male gametophytes from the plant 11.35*m* must be of two sorts (R and r). Thus in both crosses (11.35 $m \times a$ and 11.35 $a \times m$) the character of the red papillæ is represented in certain of the gametes both male and female and the character is not sex-limited.

The plants of the culture 12.44 (11.35 $a \times m$) also failed to show consistently the other differences associated with the presence or absence in the F_1 of red papillæ. There was a similar progressive advance over the parent species in leaf size and vigor, and likewise no advance in flower size, the petals ranging from 1.3–3 cm. in length.

4. *The F₂ Generation from 11.35La.*—This plant, 11.35La (Davis, '12a, pp. 401–406, Figs. 10, 11, 12 and 13), was one of the most interesting of my hybrids because of its strong resemblance to *Lamarckiana* in buds and foliage. The coloration of the stem was, however, that of Class II, *i. e.*, it was *grandiflora*-like in the absence of red in the papillæ on green portions of the stem. I had no means of knowing, when this plant was selected as the parent of a second generation, that its type of stem coloration was probably recessive to that of the red papillæ as found on the *biennis* parent, and that I should be disappointed in my hope of obtaining in an F₂ some plants with the stem characters of *biennis* D and *Lamarckiana*. I now believe that such a form is unable to produce in later generations plants with red papillæ, and, since this is an important character of *Lamarckiana*, my efforts with this particular line of hybrids will be discontinued. The F₂ generation from this plant, however, from the genetical standpoint proved to be one of the most interesting that I have grown and well merits a brief description.

The contents of 14 capsules, containing 2,217 seeds, were sown, and after eight weeks gave a culture, 12.41, of 623 seedlings. An unusual mortality, apparently in a class of dwarfs, reduced the culture finally to 532 plants. The rosettes before they were half grown presented an extraordinary range of variation and it became possible to group them although this preliminary classification required considerable revision later. A large group of more than 100 rosettes presented broad closely clustered and crinkled leaves of the *Lamarckiana* type. Many of these rosettes when half grown were indeed indistinguishable from those of *Lamarckiana* at the same age. A smaller group of about 20 consisted of rosettes with narrow leaves; most of these developed into dwarf forms. Finally, the remainder, constituting what might be called the mass of the culture, contained rosettes ranging on the one hand from a number somewhat *grandiflora*-like to a few rosettes somewhat close to the *biennis* type, and be-

tween these extremes was an assemblage of intermediates impossible of classification. In short, this portion of the culture presented an excellent illustration of a relative segregation of characters, with the extremes, however, quite far from the pure parent types. As the culture grew to maturity a reclassification of the types became necessary and finally five groups were separated as described below.

Group *A* consisted of 132 plants which had the *Lamarckiana*-like foliage and short internodes (Fig. 16) of the parent F_1 hybrid 11.35*La*, together with the 4-angled buds and flower form of this plant. These plants developed from the group of rosettes with broad crinkled leaves of the *Lamarckiana* type. The size at maturity ranged from plants 1.3 m. high to dwarfs 4 dm. in height; the habit and leaf size exhibited great variation. The extreme types of dwarfs (13 in number) had very much the habit of *nanella*. The flowers varied greatly in size, petals 3.5–1 cm. long, with the stigma both above and below the level of the anthers. There was, therefore, in this group a decided segregation of flower size. A peculiar feature of these flowers was the very common cutting of the petals at the edge into narrow segments as in laciniate varieties of flowers. This is, as far as I know, a new character in the genus *Ænothera*. The greatest development of leaf size and extent of crinkling observed in this group is illustrated in Fig. 17, which shows two rosette leaves of one of the hybrids, 12.41*Lp*, compared with the rosette leaves of the parent types of *biennis* and *grandiflora*.

Group *B* contained 5 dwarfs, 3–4 dm. high, sparsely branched or not at all, and with narrow leaves. The buds and flowers were *grandiflora*-like in form, but the petals were only about 1.8 cm. long. These dwarfs were very delicate and presented the characters of *Ænothera* reduced in size to about the simplest terms. They recalled the class of dwarfs in the F_2 from the plant

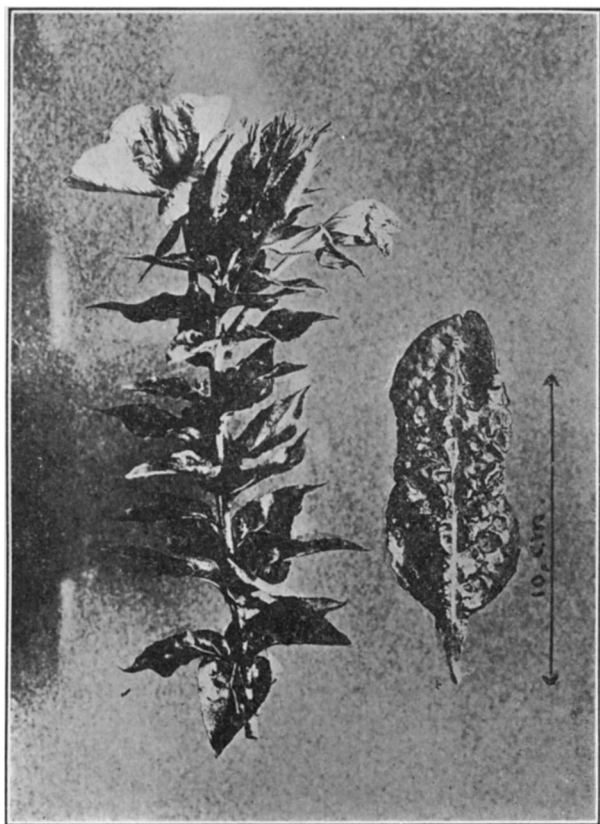


FIG. 16. A type, 12.41Li, in the F_2 from the F_1 plant 11.35La, hybrid of *grandiflora* $B \times biennis$ D, representative of Group A. A form similar to *Lamarckiana* in foliage, four-angled buds and fairly large flowers (petals 3 cm. long), but the stem coloration was the type of *grandiflora*, and the internodes were short as in *gigas*.

10.30Lb (Fig. 5), but were present in very much smaller proportions.

Group C comprised 7 plants having the habit of *grandiflora* with long branches from the base, but with narrow lanceolate leaves. The flowers were *grandiflora*-like (petals 3 cm. long), but the plants were not so high (about 8 dm.). The plants were distinguished with difficulty from certain forms in group F.

Group D consisted of 3 plants, short and very much

branched and with revolute leaves, very narrow above. The plants failed to flower.

Group *E* included 23 plants with a stiff upright habit and much-crinkled leaves. They resembled most closely the larger forms in group *A*, but were without the short internodes characteristic of those plants.

Group *F* contained the mass of the culture, 362 plants, after the separation of the groups described above. As a group it presented the best illustration of the relative segregation of characters that I have so far met in an F_2 generation. There was a very wide range of variation in flower size, habit and leaf form. A few types resembling *grandiflora* could be picked out at one end of the series, while at the other end were plants much closer to the *biennis* parent than have usually been found.

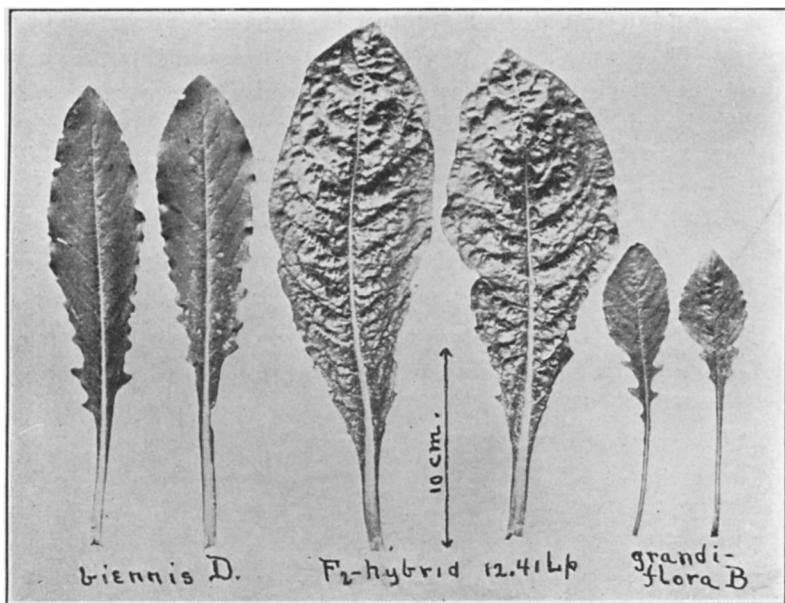


FIG. 17. Rosette leaves of a type, 12.41Lp in the F_2 from the F_1 plant 11.35La, hybrid of *grandiflora* B \times *biennis* D, representative of Group A. The rosette leaves are contrasted with those of the parents. The progressive advance in leaf size and crinkled texture is very striking. The dry weight of the two leaves of the hybrid (1.65 g.) was more than twice that of the two leaves of *biennis* and more than nine times that of the two leaves of *grandiflora*.

Curiously the tendency in this group appeared not to be progressive as to the size of flowers and other plant organs, but, instead, retrogressive. There were no plants with flowers larger than those of *grandiflora*, but in contrast a large number had flowers (petals 1–1.5 cm. long) much smaller than those of the *biennis* parent type (petals about 2 cm. long). The general tendency throughout this group, as well as that of group *A*, was distinctly downward as regards the size of the plant's organs. This is the first time that I have met with such a phenomenon in my observations on second generation hybrids of *Ænothera*.

Considering the culture, as a whole, it presented the same sort of extreme variation that has appeared in other F_2 generations. Many types were present which were taxonomically distinct from either parent of the cross and from the F_1 hybrid plant 11.35*La*. The groups of dwarfs included few individuals, but these were quite as puzzling in their extreme reduction in size as were the dwarf types described from the F_1 plants 10.30*La* and 10.30*Lb*.

4. A DISCUSSION OF THE BEHAVIOR OF THE HYBRIDS IN THE SECOND AND THIRD GENERATIONS WITH REFERENCE TO THE STABILITY OF MENDELIAN FACTORS

I wish briefly to point out what seem to me difficulties in interpreting the F_2 generations described in this paper in accordance with a strict Mendelian conception of the stability of factors. These difficulties are not presented as a criticism of Mendelism, for the data are not sufficient to justify conclusions, but it is well to note the problems.

As I understand the tenets of strict Mendelism it is assumed that the factors believed to be responsible for characters are stable. New characters are believed to appear either by the loss of factors or by their recombination in the gametes, with possibly the occasional introduction of new factors or modification of the old to give

“mutations.” The process of segregation, of course, adds or subtracts nothing from the sum total of the factors but merely distributes them variously to the gametes that are formed. The increase or loss of factors in the offspring of a hybrid results from the mating of gametes which carry a greater or less number of factors.

Mendelism in its extreme expression may then be said to rest in large part on a law of *the conservation of factors*. This means that factors could never disappear from a genetic line of development if all of the gametes were mated and if all of the zygotes matured. It follows that the factors contained in an F_1 hybrid must all come out in an F_2 generation if that generation is sufficiently numerous.

The most striking specific problems brought forward by the data presented in this paper are:

1. The explanation of the large groups of dwarfs thrown off in the F_2 generations and repeated by certain plants in the F_3 .
2. The explanation of the well-defined progressive evolution, excluding the dwarfs, exhibited by these same cultures.

With respect to the dwarfs the ratio of their production in the most striking of the F_2 generations is as follows:

The F_1 hybrid 10.30*La* gave 141 dwarfs in a culture of 1,451 plants (ratio about 1:9).

The F_1 hybrid 10.30*Lb* gave 147 dwarfs in a culture of 992 plants (ratio about 1:5.7).

These are large ratios, considerably above the 1:15 which might be expected if the range of size depended upon so simple a matter as the presence or absence of two factors. It must be remembered that the dwarfs were very much smaller than either parent, as best shown in the dwarfs from the plant 10.30*Lb* (Fig. 5), where the proportion, about 1:5.7, was the largest. These small plants (Fig. 5), 3–4 dm. high, came from parents, *biennis* and *grandiflora*, about 10–15 dm. and 15–20 dm.

high, respectively. It is difficult to imagine fertile hybrids of such parentage much more reduced in their vegetative expression than are these dwarfs. Furthermore, the reduction was apparently a complete loss in the power of a greater growth, as was indicated by the dwarfs breeding true in the F_3 generation.

If the dwarfs were to be interpreted in so simple a manner as recessives from a cross where two factors for size were allelomorphic to their absence the ratio of the dwarfs to the mass of the culture should have been as 1:15. Why then in the mass of the culture, dwarfs excluded, was there no evidence of other classes? The two factors assumed must be of large value if their absence is to make the difference between the size of the dwarfs, 3-4 dm., and the size of the parents, an average of about 15 dm. There might be expected a class of giants to balance the class of dwarfs and in the ratio of 1:15. There should have been several other classes ranging between these giants and the dwarfs. With only two factors for size concerned, and these of such large value, it seems impossible that the fluctuating variations could conceal the presence of such classes. Yet the mass of the culture failed to exhibit them, and only the dwarfs could be separated as a class sufficiently distinct to warrant its designation. The mass of the culture ranged in size approximately between the limits of the parents; the gap between them and the dwarfs was not bridged by intermediates.

I am aware that the dwarfs might be explained as resulting from the presence of an inhibiting factor introduced into the cross, but again there should have been evidence of other size classes together with the dwarfs according as the inhibitor was present in a full or in a lessened amount or was entirely absent. These difficulties are in themselves of sufficient weight, let alone the general improbability of such a situation.

The explanation of the progressive evolution of an F_2 generation in which the culture with respect to cer-

tain characters appears to advance as a whole presents the second problem to be considered. This phenomenon was also best exhibited by the F_2 generations from the hybrids 10.30*La* and 10.30*Lb*. A large number of plants in these cultures bore flowers with petals 1 cm. longer than those of the *grandiflora* parent (petals about 3.3 cm. long), and the smallest flowers were, for the most part, two or more times larger than those of the *biennis* parent (petals about 1.3 cm. long); between these extremes was a very perfect range of intermediates. An explanation for the advance in flower size over that of *grandiflora* may, of course, be offered as a recombination of factors for large size on the hypothesis of multiple factors for the size of petals, but why was there not a balancing group of plants with flowers as small as or smaller than those of *biennis*? Even the dwarfs of these cultures had flowers larger than those of the *biennis* parent. The only plant having smaller petals was the extraordinary form 11.42*j* (Fig. 15). What had become in these cultures of the factors responsible for small size?

A similar situation was presented by the character of the foliage most markedly exhibited by the F_2 generations from 10.30*La* and 11.35*a*. The leaves throughout the mass of these cultures were much larger than those of the parents of the cross and much more crinkled. There was thus a marked progressive advance in leaf size with the absence of small-leaved classes of plants unless such were represented in the F_2 from 10.30*La* by the dwarfs. Admitting that possibility, the same problem must be faced as was discussed for the explanation of the dwarfs themselves which were present in a ratio of about 1:9, suggesting the 1:15 ratio with the presence of two factors for leaf size. Thus two factors for leaf size should give through the culture other classes besides those of the recessives, and these were not evident. Applying the hypothesis of multiple factors for leaf size one is compelled to enquire what has become of the fac-

tors or combination of factors that should give classes of small-leaved plants to balance the mass of the culture with its progressive advance in leaf size and degree of crinkling.

I present these problems not altogether as a criticism of the hypothesis of multiple factors which has been so ably applied in the recent *Oenothera* study of Heribert-Nilsson ('13), and by East, Hays and other investigators in various groups. This hypothesis has amply justified the confidence of its advocates, but it does not seem to me to be established as wholly satisfactory. There has been abundant evidence in my cultures of a segregation of size in the F_2 , but my question is whether this segregation may not be accompanied by a modification of factors whereby new sets wholly, or in part, take the place of the old. I do not think that East ('12) quite met the problem in his recent discussion of my data.

It has been suggested to me that the marked progressive advance in the size of organs in an F_2 generation may result from the continuance of the stimulus of heterozygosis (East and Hays, '12) apparent in the F_1 . Is it not, however, possible to view the phenomenon in the F_2 as the direct modification of the factors for size as a result of the cross? One of the most extreme illustrations that I have observed of such an advance is illustrated in Fig. 17, which shows rosette leaves of a certain F_2 hybrid plant (12.41*Lp*) in comparison with those of its parents. This plant failed to mature flowers and its study could not be continued. There was certainly indicated very profound changes in its vegetative organization.

Advocates of the hypothesis of multiple factors for size allelomorphic to their absence may claim the possibility of selective fertilization in the formation of zygotes preceding an F_2 or later generations. This possibility can not be disregarded, but we have no data for the *oenotheras*. There has been, however, in my experience usually a high degree of sterility in the seeds of *Oeno-*

thera hybrids following the F_1 for which no adequate explanation is known.

In one F_2 generation I have noted a distinct retrogression in the size of the flowers. This was the F_2 from the plant 11.35*La* briefly described in this paper. It contained no plants with flowers larger than those of *grandiflora* and a large proportion of the culture bore flowers as small as or smaller than the flowers of the *biennis* parent. The *biennis* parent in this cross was a rather large-flowered type (*biennis D*, petals about 2 cm. long) which made the retrogression appear the more marked.

A striking feature of the F_2 generations here considered has been the diverse progeny from F_1 sister plants of the same culture. Thus the F_1 hybrids 10.30*La* and 10.30*Lb* were sisters of the cross *grandiflora B* \times *biennis A* and the F_1 hybrids 11.35*m*, 11.35*a* and 11.35*La* were sister plants of the cross *grandiflora B* \times *biennis D*. Each plant gave its own peculiar set of types in the F_2 with peculiarities so pronounced that the blood relationship was much obscured. This is difficult to understand except on the theory that the parent stock was heterozygous; yet there has appeared no evidence of this in the cultures of the pure species. It is, however, clear that I have been working with complex material and it is not certain that the species of *Ænothera* employed in my crosses have been homozygous to the degree demanded for experimentation on the behavior of unit factors. For this reason I have endeavored to discuss the problems with full caution and I hold my point of view tentatively.

5. THE HABIT OF "MUTATION" IN *Ænothera Lamarchiana* DE VRIES CONSIDERED WITH REFERENCE TO THE
BEHAVIOR OF THE HYBRIDS BETWEEN
biennis AND *grandiflora*

Perhaps the most important observations on these hybrids of *grandiflora* and *biennis* in the second and third generations have been those showing a close parallelism

of their behavior to that of *Oenothera Lamarckiana*. Thus the hybrids have thrown off marked variants of new specific rank as does *Lamarckiana*. Certain of these new forms have held true and others have continued to throw variants as do some of *Lamarckiana*'s "mutants." One form (12.56*x*) appeared with a marked increase over the normal chromosome number (14) and apparently corresponds closely to the triploid "mutants" from *Lamarckiana* or its derivatives (Lutz, '12; Stomps, '12*a*). A most striking feature has been the production in successive generations of classes of dwarfs, plants which contrast sharply with the mass of the culture and which are stable.

This behavior of the hybrids appears to me to be of quite the same character as the "mutations" of *Lamarckiana*, but the results, here concerned with crosses between distinct species, are clearly of the sort that were to be expected from their hybrid association. It is not fundamental to my position that the various forms of the variants in the F_2 and F_3 generations should match the "mutants" from *Lamarckiana*. Since the F_1 hybrids were not themselves the counterpart of *Lamarckiana*, they should not be expected to give the same progeny as this latter plant. It is sufficient for my purpose to point out the essential parallelism between this hybrid behavior and that of *Lamarckiana* when it gives rise to its "mutations."

De Vries ('12, p. 30) has questioned the stability of my *grandiflora* stock, apparently believing that my hybrids exhibit, at least in part, a mutating habit inherited from the *grandiflora* parent. This view is based on the appearance of two classes of hybrids (twin hybrids) in the F_1 from the cross *grandiflora* *B* \times *biennis* *D*. The evidence, however, indicates that this peculiarity is connected with the *biennis* parent, which may not have been homozygous for the character of stem coloration at the time the cross was made, although in later generations the form has held true.

I am perfectly willing to admit the complexity of my stock material of *grandiflora* and *biennis*, and also the possibility that the forms may not have been strictly homozygous at the time the crosses were made. It was in no wise necessary for the purposes of my experiments that they should be strictly homozygous. My only concern was that the material should be American types of *Ænothera* without the possibility of contamination through crosses with *Lamarckiana*. That my forms of *biennis* and *grandiflora* had these qualifications there can, I think, be no doubt. They have, as a matter of fact, bred true in the small cultures which have been carried through two generations for *biennis A* and *biennis D* and four generations for *grandiflora B*.

An abstract of my argument is as follows: (1) Since hybrids of *biennis* and *grandiflora* show points of strong resemblance to *Lamarckiana* and, (2) since the behavior of these hybrids in the F_2 and F_3 parallel closely the behavior of *Lamarckiana* when it gives rise to "mutants," (3) therefore, there are strong reasons for believing that the "mutations" of *Lamarckiana* are due to instability of its germinal constitution resulting from a hybrid origin. The fact that *Ænothera Lamarckiana* is not known as a component of any native *Ænothera* flora and the fact that its known history has been entirely as a cultivated plant or as a garden escape naturally greatly strengthen the force of the above argument.

It does not seem to me that these arguments are answered by a supposition that the behavior of my hybrids involves a habit of mutation inherited from the parental types. On the contrary, are we not justified in asking of the mutationists evidence from material the status of which, as representative of a wild species, is beyond question? Stomps ('12b) has apparently endeavored to meet the situation by a study of a cross between the *biennis* and *cruciata* of the sand dunes of Holland. From the cross he obtained in the second generation a *biennis nanella* and a *biennis semi-gigas*. Both of

these new forms are regarded by Stomps as "mutants" in the De Vriesian sense in the belief that *biennis* and *cruciata* have an identical germinal constitution, except for factors that determine floral structure and, therefore, with respect to other characters may be crossed as though they were homozygous. Applying these conclusions to the problem of the status of *Æ. Lamarckiana*, Stomps reasons that since *biennis* mutates and since *biennis* is an older species than *Lamarckiana*, it follows that mutations among the *œnotheras* are older than *Lamarckiana* and consequently the mutations of this species can not be the result of hybridization.

In a recent discussion (Davis, '13) of the conclusions of Stomps I have taken exception to the assumption that his *biennis* and *cruciata* have exactly the same germinal constitution except for floral characters. This I can not believe probable, for the reason that, whatever may be the relation between the two species, they have certainly had a long period of independence. *Cruciata* has never appeared in the extensive cultures of the Dutch *biennis* that have been grown by De Vries and Stomps, and there is no experimental evidence that it has been recently derived from the latter form. From my point of view Stomps really made a cross between two species and obtained two marked variants due to some germinal modification as a result of the cross.

It seems to me fair to ask: Why did Stomps find it necessary to cross *biennis* and *cruciata* to obtain these "mutants" *biennis nanella* and *biennis semi-gigas*? If they have the same germinal constitution except for floral characters, Why should not *biennis* alone or *cruciata* alone give the "mutants"? There is no form of *biennis* better known to the workers in the experimental gardens than this Dutch plant. It is believed to have been on the sand dunes of Holland since pre-Linnæan times and Bartlett ('13) has recently presented strong reasons for believing the plant to be the form known to Linnæus as *Ænothera biennis* and consequently to be regarded as

the type-form of the species. No species of *Ænothera* is perhaps so free from suspicion as to its gametic purity. If Stomps can obtain mutations from tested material of the Dutch *biennis* grown in pure lines he will have the basis of a strong argument, but this seems to me lacking in the conclusions drawn from his cross of *biennis* with *cruciata*.

I do not believe it at all probable that the Dutch *biennis* will be found to "mutate" under normal conditions to a degree worthy of serious consideration for the mutation theory of De Vries. The plant has already been made the subject of extensive cultures and its characters are known to a number of workers with *œnotheras*. Yet I am far from taking the stand that environmental conditions may never induce a modification of germinal constitution and still leave the organism vigorous. The possibility of direct modification of germ plasm, independent of sexual mixing, presents one of the most inviting fields of genetical research. However, if such research gives affirmative conclusions we should be most cautious in applying them to the conditions that normally surround a species and to the process of organic evolution.

6. THE PROBLEM OF THE ORIGIN OF *Ænothera* *Lamarckiana* DE VRIES

As stated in the introduction to this paper, we are no longer in our problem of the origin of *Ænothera Lamarckiana* De Vries concerned with Lamarck's plant (*Æ. Lamarckiana* Seringe, 1828) of about 1796. This plant (Davis, '12b) was with little doubt a form of *Æ. grandiflora* Solander, 1789, introduced at Kew in 1778. It had no relation to the cultures of Carter and Company, of about 1860, which were the starting point for the distribution among seedsmen of the plants known in cultivation as *Lamarckiana* (an incorrect determination of Lindley) from some of which De Vries's material was derived.

The historical side of the problem then largely centers on the history and composition of these cultures of Carter and Company. We have the statement of this firm that their seeds were received unnamed from Texas. This suggests that *Lamarckiana* De Vries may have in it the blood of some of the large-flowered *œnotheras* with an upright habit that are known to be present in the southwestern United States. There are a large number of such forms which frequently pass under the name of *œnothera Hookeri* and have not as yet been properly segregated in the experimental garden. I am working with several of these types to determine whether any of them may prove to be more favorable than *grandiflora* as forms to cross with *biennis* in my attempts to synthesize *Lamarckiana* as a hybrid. (See note at end of paper.)

It must, however, be borne in mind that we have at present no confirmatory evidence that such plants as Carter and Company describe or the *Lamarckiana* of De Vries's cultures grow in Texas. It is possible that Carter and Company obtained their plants from some part of England, as from the sand hills of Lancashire, where large-flowered *œnotheras* were reported at dates earlier than 1860 and where at the present day *œ. Lamarckiana* is successfully established. We must look to British botanists for investigations which will make clear the history of such *œnothera* floras as that of Lancashire, and it is to be hoped that collections will be thoroughly searched for evidence on their probable development.

With respect to the composition of the cultures of Carter and Company we have some strong evidence from the specimens grown by Asa Gray in 1862 that their plants differed in some important respects from the *Lamarckiana* of De Vries. These specimens have been figured and described (Davis, '12a, pp. 417-422) and it seems probable that the plants were not more than one or two generations removed from the original cultures of Carter and Company. The specimens have characters in part those of De Vries's *Lamarckiana* and in part those

found in *grandiflora*, and undoubtedly present in some of the large-flowered *ænothëras* of the west and southwest. If the plant of Dr. Gray was representative of the cultures of Carter and Company the evidence indicates that their forms became greatly modified during the quarter century before the time when De Vries began his studies, at about 1886, and isolated the type which we know to-day as *Ænothera Lamarckiana* De Vries.

On the experimental side of the problem of the origin of De Vries's *Lamarckiana* we have evidence of its hybrid nature from various sources. The recent analytical studies of Heribert-Nilsson ('12), previously mentioned, show that certain characters of *Lamarckiana* behave in a manner suggesting their association in a complex hybrid that is still throwing off segregates. His conclusions that *Lamarckiana* is a polymorphic species is supported by my own experience in isolating certain biotypes from material of De Vries. The "twin hybrids" produced when *Lamarckiana* or certain of its derivatives furnish the pollen of a cross with *biennis* or *muricata* indicate, as suggested by several critics, that different types of gametes are formed by *Lamarckiana*.

My own studies on hybrids between forms of *biennis* and *grandiflora* have reached an interesting point. I have not been able to synthesize by direct crosses, from wild stock so far obtained, any hybrid with all of the characters of *Lamarckiana* in the same plant, although I believe that all of the important taxonomic characters of *Lamarckiana* have been represented in some of my hybrids. It is, however, probable that more favorable parental types will in time come to hand. For example, a form, with the habit and foliage of the Dutch *biennis* and with the stem coloration of *Lamarckiana*, which the Dutch *biennis* apparently has not, would furnish very favorable material. In the meantime I have the possibility of crossing my hybrids back with certain wild species and of crossing the hybrids with one another. In this way it may be possible to bring into one plant all of

the characters of *Lamarckiana*. It is of course in no way essential to the hypothesis of the hybrid origin of *Lamarckiana* that the plant should have arisen as the product of a simple cross. With Lamarek's plant eliminated from the problem of the origin of De Vries's material, the importance of *grandiflora*, on historical grounds, is materially lessened and we may consider other large-flowered types of more recent introduction into Europe as possible parents in a cross.

The resemblance of my various hybrids to *Lamarckiana* and the parallelism of their behavior in the F_2 and F_3 to that of *Lamarckiana* give in themselves sufficient reasons, in my opinion, to justify the belief in its hybrid character and to point to the probability that this plant arose as a cross between distinct forms of *Oenothera*. *Lamarckiana* thus would not be representative of a wild species of essentially stable germinal constitution and its "mutations" are most simply interpreted as the behavior of a hybrid.

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It is a satisfaction to announce that this summer (1913) I have obtained an F_1 hybrid generation with, I believe, all of the essential taxonomic characters of the small-flowered forms of *Æ. Lamarckiana* De Vries. The cross was a large-flowered, undescribed species of *Ænothera* from California pollinated by the Dutch *biennis* (*Æ. biennis* Linnæus). The hybrids differ from the small-flowered *Lamarckiana* only in relatively small plus or minus expressions of certain of its distinctive characters. It is not unreasonable to expect that generations from these hybrids in the F_2 will give material for future selection towards the large-flowered *Lamarckiana* of De Vries.